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Prevalence of nine different micro-organisms in the female genital tract

A comparison between women from a venereal disease clinic and from a health control department

K. PERSSON,* KRISTINA PERSSON,† H. HANSSON,‡ B. BJERRE,‡ L. SVANBERG,‡ T. JOHNSSON,* AND A. FORSGREN†

*From the Departments of *Clinical Virology,† Clinical Bacteriology,‡ Dermatovenereology and ‡Gynaecology, Malmö General Hospital, Malmö, Sweden*

SUMMARY In a study of the prevalence of nine different micro-organisms in the female genital tract in a Swedish population, significantly higher isolation rates occurred among women attending a venereal disease clinic than among those attending a gynaecological health control department. The prevalence of *Candida albicans,* however, was similar in different groups, individual susceptibility being the most important factor. *Chlamydia trachomatis, Trichomonas vaginalis,* and *Mycoplasma hominis* occurred concomitantly with *Neisseria gonorrhoeae,* indicating a similar epidemiology for all these agents. Younger patients seemed to have an increased susceptibility to *C. trachomatis* whereas older patients had an increased susceptibility to *T. vaginalis.*

Introduction

Besides *Neisseria gonorrhoeae* genital symptoms can be caused by *Trichomonas vaginalis, Chlamydia trachomatis* (Dunlop *et al.,* 1972), *Mycoplasma hominis* (Mårdh *et al.,* 1975), *Candida albicans,* and herpes simplex virus. *Ureaplasma urealyticum* has not been connected with symptomatic infections in the female genital tract and its role in non-specific urethritis in men is still undecided, although recent findings have again indicated its role as a potential pathogen (Bowie *et al.,* 1977). Cytomegalovirus causes foetal damage and group B streptococci may cause neonatal infections, but neither has been associated with genital symptoms. We have investigated the occurrence and interrelationship of nine different micro-organsims found in the female genital tract. The isolation rates of a group of patients attending a department of venereal diseases were compared with those of a group of women attending the gynaecological health control department and with those of a group of young healthy girls seeking contraceptive advice.

Address for reprints: Dr K. Persson, Department of Clinical Virology, University of Lund, Malmö General Hospital, S-21401 Malmö, Sweden

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Patients and Methods

STUDY POPULATION

The present investigation included three groups of women.

Group 1

All untreated women were examined for the presence of *N. gonorrhoeae* and *C. trachomatis* at their first visit to the venereal disease clinic during 1977. The group consisted of 755 women, with a median age of 23 years and a range of 13-70 years. Three per cent of this group were immigrant women of European origin.

Additional cultures (390) were performed during the period February to June for *T. vaginalis, M. hominis, U. urealyticum,* group B streptococci, and *C. albicans.* Between August and October cultures were performed for herpes simplex virus and cytomegalovirus (319). Of the entire study population, 53 women were consorts of men who had positive culture results for both *N. gonorrhoeae* and *C. trachomatis.*

Group 2 (controls)

All women attending the gynaecological health control department, which covers over 90% of the

female population over 20 years of age, were examined for all nine micro-organisms during the period March to May. The group consisted of 201 women, with a median age of 26 years and a range of 23-30 years. About 13% were immigrant women of Finnish and Slavic origin, which is close to the average figure for the whole of Sweden.

Group 3 (controls)
A group of 111 young healthy girls seeking contraceptive advice at the department of gynaecology was examined for the presence of *N. gonorrhoeae, C. trachomatis*, herpes simplex virus (HSV), and cytomegalovirus during the period from September to October. The age of these girls ranged from 12 to 19 years, with a median age of 16 years. Two per cent of the group were immigrants.

ISOLATION PROCEDURE
Chlamydia
Specimens were obtained from the cervix with a cotton-wool swab, which was placed in modified 2 SP transport medium, in which 10% sorbitol had been substituted for sucrose (Richmond, 1974). Irradiated McCoy cell cultures were used and chlamydial inclusions were detected microscopically after being stained with iodine.

Herpes simplex virus and cytomegalovirus
The same specimen used for chlamydial isolation was also examined for herpes simplex virus and cytomegalovirus. Human embryonic lung fibroblast cultures were inoculated and positive isolates were identified as herpes simplex virus by a complement fixation (CF) test and as cytomegalovirus by direct immunofluorescence.

Group B streptococci
Cotton-wool swabs from the urethra and rectum were placed in tubes containing a selective broth medium (Baker *et al.*, 1973). Suspicious organisms were identified as group B streptococci by serotyping (Christensen *et al.*, 1973).

Yeasts and T. vaginalis
Material was taken from the vaginal fornix, put into a tube with Diamond's broth (Diamond, 1957) and examined microscopically on days 3, 4, and 5.

Neisseria gonorrhoeae
Urethral, rectal, and cervical specimens were inoculated at the bedside on two chocolate-ascites agar plates, and positive isolates were confirmed by fermentation tests (Juhlin, 1963).

Mycoplasma hominis and Ureaplasma urealyticum
Modified Shepard agar and a broth of modified Shepard medium with urea were inoculated at the bedside. The diagnosis of *M. hominis* was made microscopically, and *U. urealyticum* was identified by its ability to hydrolyse urea and by its microscopical appearance (Mårdh *et al.*, 1975).

All plates and tubes were immediately transported to the laboratories and were processed within four hours.

Statistical comparisons were made using the χ^2 test throughout.

Results

Details of the isolation rates for the different agents are shown in Table 1. Significantly higher rates were found in the study group (group 1) than in the control group (group 2) for all micro-organisms except *Candida*, for which the difference was not statistically significant. In control group 3 *N. gonorrhoeae* was found in one patient, *C. trachomatis* in nine, and cytomegalovirus (CMV) in

Table 1 *Isolation rate for each of nine different micro-organisms*

	Group 1 (study group)				*Group 2 (control group)*	
Micro-organism	*No. of cultures*	*% isolation-positive for each agent*	*% of N. gonorrhoeae-positive cultures with positive isolation for other agents*	*% of N. gonorrhoeae-negative cultures with positive isolation for other agents*	*No. of cultures*	*% isolation-positive for each agent*
N. gonorrhoeae	755	28			200	0·5
C. trachomatis	755	25	40	20	201	5
M. hominis	382	41	53	37	200	19
T. vaginalis	390	12	20	9	201	1
U. urealyticum	382	75	74	76	200	60
Group B streptococci	379	41	40	42	189	28
Cytomegalovirus	319	7	9	7	201	1
Herpes simplex virus	319	4	7	4	201	0·5
Yeasts	390	25	19	27	201	22

Statistical difference between study group and control group for *N. gonorrhoeae, C. trachomatis, M. hominis, T. vaginalis*, and *U. urealyticum*, $P<0·001$; for group B streptococci, CMV, and HSV, $P<0·01$; and for yeasts, $P>0·05$

four, which is a significantly lower rate than in group 1. Associations between *N. gonorrhoeae* and *C. trachomatis* (P<0·001), *T. vaginalis* (P<0·01), and *M. hominis* (P<0·01) were evident in group 1 and could not be analysed in the control groups, as the number of positive isolates was too small.

CONCOMITANT INFECTIONS

In the study group (group 1) 40% of patients harbouring *N. gonorrhoeae* also harboured *C. trachomatis* compared with 20% of the patients without gonorrhoea; similarly, 53% harboured *M. hominis* and 20% *T. vaginalis* of those patients who also harboured *N. gonorrhoeae* compared with 37% and 9% respectively of those patients without gonorrhoea (Table 1). No correlation was found between the isolation of *N. gonorrhoeae* and that of group B streptococci, *U. urealyticum,* HSV, CMV, or yeasts.

Of the nine different micro-organisms studied, on average three or four were detected among those patients in group 1 who harboured *N. gonorrhoeae* (Fig. 1). The mean number of isolated micro-organisms was highest for patients who harboured *N. gonorrhoeae.* A lower mean number was found for those patients who harboured *C. trachomatis,* and the lowest mean number occurred among those with concomitant infection with *U. urealyticum.*

AGE FACTOR

The prevalence of *N. gonorrhoeae* and *C. trachomatis* in the study group (group 1) was the same, with a peak around 20 years (Fig. 2). The prevalence of *N. gonorrhoeae* and *C. trachomatis* for the different ages in this group are also shown in Table 2. The isolation rate for *N. gonorrhoeae* in the six age groups did not show a significant difference between the groups by the χ^2 test. The rate for *C. trachomatis* for the same age groups, however,

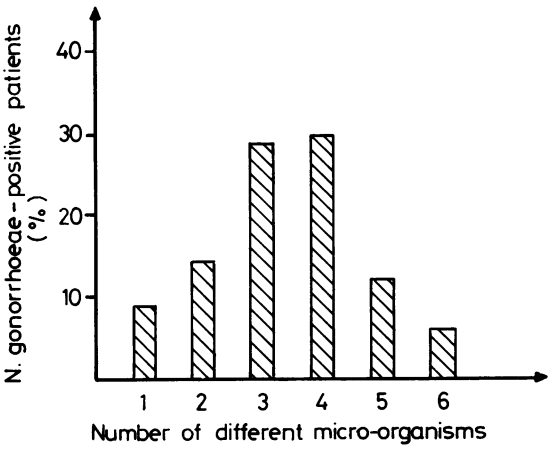

Fig. 1 *Percentage of patients harbouring N. gonorrhoeae in the study group in relation to the number of agents isolated*

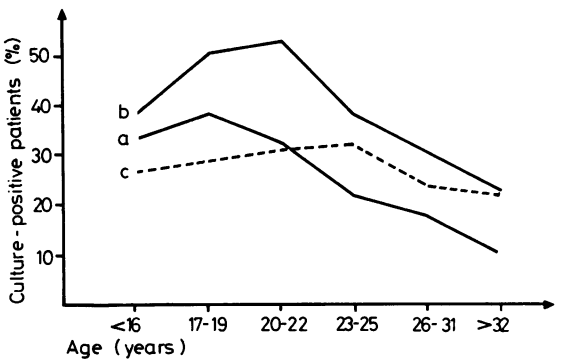

Fig 2 *Distribution of N. gonorrhoeae and C. trachomatis for different age ranges of the study group (group 1): (a)—— C. trachomatis in group 1; (b)—— C. trachomatis in patients with gonorrhoea (group 1); (c)- - - N. gonorrhoeae in group 1*

Table 2 *Isolation rate for three different micro-organisms compared with that for N. gonorrhoeae in different age ranges for study group (group 1) and two further groups of patients attending the venereal disease clinic (groups 1a and 1b)*

	Group 1	*Positive isolation results (%) for*		*Group 1a*	*Positive isolation results (%) for*		*Group 1b*	*Positive isolation results (%) for*	
Age groups	*No. of patients*	*C. tracho-matis**	*N. gonor-rhoeae†*	*No. of patients*	*M. hom-inis‡*	*N. gonor-rhoeae§*	*No. of patients*	*T. vagi-nalis*	*N. gonor-rhoeae*
Under 16	48	33	27	34	41	21	34	3	21
17-19	161	38	29	69	38	26	73	3	26
20-22	139	32	27	71	30	25	72	11	29
23-25	146	22	32	77	44	32	79	18	34
26-31	139	18	24	64	47	17	62	15	16
32 and over	122	10	22	61	48	31	64	19	28
Total	755	25	28	376	41	26	384	12	27

Stastical differences: *C. trachomatis,* $\chi^2 = 28·81$, P<0·001; *N. gonorrhoeae,* $\chi^2 = 3·23$ (group 1), $\chi^2 = 4·16$ (group 1a), $\chi^2 = 4·97$ (group 1b); *M. hominis,* $\chi^2 = 3·81$; *T. vaginalis,* $\chi^2 = 12·54$, P<0·05

exceeded chance variation between the groups ($P<0.001$).

The same statistical comparison was made for 376 patients (group 1a) for whom isolation results were available for both *M. hominis* and *N. gonorrhoeae* (Table 2). The age groups did not differ significantly from each other for the isolation of either *M. hominis* or *N. gonorrhoeae.*

A group of 384 patients (group 1b) with isolation results for both *T. vaginalis* and *N. gonorrhoeae* was also analysed (Table 2). The isolation rate for *T. vaginalis* differed in different age groups to a statistically significant degree ($P<0.05$). The rates for *N. gonorrhoeae* were within the limits of normal variation. Thus, there was a higher likelihood of young patients harbouring *C. trachomatis* than older ones and a higher likelihood of *T. vaginalis* occurring in the older age groups. The isolation of *N. gonorrhoeae* and *M. hominis* was evenly distributed and did not occur more frequently in any particular age group.

Of the 53 female consorts of men who harboured both *N. gonorrhoeae* and *C. trachomatis*, 43 (81%) harboured *N. gonorrhoeae* and 23 (50%) harboured *C. trachomatis*. Twenty consorts harboured both agents. Thus, the isolation rate for *C. trachomatis* was significantly lower than that for *N. gonorrhoeae* ($P<0.001$) in these patients.

Discussion

In this study all agents except yeasts were found significantly more often among patients attending the venereal disease clinic (group 1) than among those attending the health control department (group 2). *C. trachomatis, T. vaginalis,* and *M. hominis* were strongly associated with *N. gonorrhoeae,* which would suggest that they have a similar epidemiology and are sexually transmitted. Reactivation of a latent chlamydial infection has been suggested by Richmond and Sparling (1976), which could also be manifested as a statistical correlation with *N. gonorrhoeae.* The study group patients harbouring *N. gonorrhoeae* had the highest mean number of isolated micro-organisms, which could indicate that these patients were the most heavily exposed to all of the six agents.

A higher prevalence of *U. urealyticum* and group B streptococci in the study group than in the healthy control group might occur for different reasons. Sexual transmission cannot be ruled out but would seem to be of minor importance, since the high prevalence of these organisms in the study group might be due to the selection of patients in this group. These saprophytic organisms seem to be carried in the female genital tract. No association between group B streptococci and *N. gonorrhoeae* was found, which agrees with earlier reports (Wallin and Forsgren, 1975).

CMV was found more frequently in the study group than in the other groups. Sexual transmission of CMV has been suggested (Jordan *et al.,* 1973) and as virus secretion might continue for months and even years a higher rate of isolation in the study group would be expected. HSV was also found more often in the study group. Sexual transmission is a likely cause of primary infection (Beilby *et al.,* 1968). Recurrence of HSV infection is common and may be initiated by another genital infection. The occurrence of HSV infection is probably an effect of both sexual transmission and reactivation of a latent infection possibly stimulated by some concomitant infectious agent.

Yeasts did not occur significantly more often in either the study group or the healthy control group. Comparable results have been reported by others (Hilton *et al.,* 1974). The occurrence of yeasts was not related to the epidemiology of venereal disease agents such as *N. gonorrhoeae* and *C. trachomatis.* Colonisation by yeasts thus seemed to depend to a great extent on the individual susceptibility of the patients, in whom local pH values, hormonal factors, and microbial competition might play a part.

The age distribution of *C. trachomatis* and *T. vaginalis* was uneven. If these agents circulated freely in the different age ranges the results would indicate an increased susceptibility for *C. trachomatis* in younger women, who might perhaps develop an immunity later, and an increased susceptibility to *T. vaginalis* in the older age group.

C. trachomatis was found significantly less frequently than *N. gonorrhoeae* among female consorts of men who harboured both agents. The isolation techniques for these two micro-organisms are unlikely to be equally sensitive, which partly accounts for the difference in prevalence. Other factors such as differences in infectivity and susceptibility might also have influenced the results.

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